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Determination Of Kinetic Parameters For The Thermally Induced Isomerization Of Methyl Linolenate

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ABSTRACT
Determination of activation energy and the order of reaction for the thermally induced isomerization reaction of Methyl Linolenate have been studied by Gas chromatography and Infra-red spectroscopy. Methyl linolenate samples have been thermally induced in sealed glass ampoules at 225, 250 and 275˚C. The ampoules under thermal induction have been removed at regular time intervals. A part of the sample from each ampoule was used for infrared spectroscopic analysis. The rest of the sample was diluted in 1.5 ml heptane and analysed by gas chromatography (GC). The products in the GC analysis were identified by using reference standards and literature references. Peak heights in the chromatograms were used in the quantification of the isomers. Each isomer was quantified as percentage in the thermally induced mixture. The activation energy was determined with respect to methyl linolenate. The concentration of methyl linolenate in the samples formed the basis for the calculation of activation energy for the isomerization reaction. The results show that the prolonged thermal induction leads to the formation of all the isomers of linolenic acid. Furthermore, the isomerization reaction followed a first order reaction with an activation energy of 24.7±0.1kcal/mol.

KEYWORDS: Linolenic acid, methyl linolenate, trans fatty acids, gas chromatography, Infrared spectroscopy

INTRODUCTION

Edible oils are glycerides of fatty acids. Most of the edible oils contain fatty acids ranging from 12-18 carbon to other fatty acids nd polyunsaturated fatty acids such as linoleic and linolenic acids found in vegetable oils are all in the *cis* configurations. However, small amounts of these fatty acids can undergo isomerization during deodorization [1]. The presence of *trans* fatty acids in food is believed to have negative health effects [2-7].

The use of cooking oils in the deep fat frying in the preparation of food has increased significantly in the modern day living [8]. During heating and frying process the unsaturated fatty acids undergo isomerization and chemical reactions resulting in the formation of *trans* isomers and several other toxic compounds [9]. During heating, the oleic acid (9c) forms one trans isomer (9t), linoleic acid (9c12c) forms three trans isomers (9t12c, 9c12t and 9t12t) and linolenic acid (9c12c15c- α-linolenic acid) forms seven trans isomers (9t12c15c, 9c12t15c, 9c12c15t, 9t12t15c, 9c12t15t, 9t12c15t and 9t12t15t). Studies of the thermal effect on the unsaturated fatty acids would give us a clear picture of the stability of the *trans* isomers formed.

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Table 1: Concentration of C18 unsaturated fatty acids in vegetable oils [10]

Vegetable oils	Composition of unsaturated C18 fatty acids in vegetable oils (%)		
	18:1 (9c)	18:2 (9c12c)	18:3 (9c12c15c)
coconut	5.4-8.1	1.0-2.1	0.0-0.2
palm	36.7-40.9	9.4-12.1	0.1-0.4
corn	24.6-42.2	39.4-60.4	0.7-1.4
Peanut	36.4-67.1	14.0-43.0	0.0-0.1
olive	55-83	3.5-21.0	0.0-1.5
Soybean	17.7-25.1	49.8-57.1	5.5-9.5
Safflower seed	8.4-21.3	67.8-83.2	0.0-0.1
sunflower	13-40	40-74	0.0-0.3

The studies of *cis-trans* isomerization in oleic and linoleic acid and determination of their kinetic parameters have been reported in the literature [10, 11]. Christy and co-workers have successfully studied thermally induced isomerization kinetics of the oleic, linoleic, and conjugated linoleic acids (9c11t and 10t12c) in triacylglycerols by FT-IR spectrometry aided by gas chromatography [10-16]. However, reports dealing with the determination of kinetic parameters for the isomerization reaction of linolenic acid are rare to be found. Wolff [17] has reported the heat induced geometrical isomerization of α-linolenic acid and found that the disappearance of α-linolenic acid follows a first order kinetics at high temperatures and Linolenic Acid Geometrical Isomers (LAGI) increased with increasing temperature and time. O'keefe and co-workers [18] have reported the effects of temperature on linolenic acid loss and 18:3 Δ9-*cis,* Δ12-*cis* and Δ15-*trans* formation in soybean oil.

Our aim in this paper is to study the *cis-trans* isomerization of methyl linolenate and determine the kinetic parameters for the isomerization reactions with respect to methyl linolenate. Methylated linolenic acid was used as the starting material to simplify the experimental procedure. Since methyl group in methyl linolenate is far away from the double bonds in the C18:3 molecule, the activation energy for the isomerization reaction should be the same as the activation energy for the isomerization reaction in linolenic acid. The present work uses the analytical techniques such as infrared spectrometry and gas chromatography to study the formation of geometrical isomers and the determination of kinetic parameters for the isomerization reaction.

Experimental:

MATERIALS AND METHODS

Methyl linolenate (≥99%), heptane (analytical grade) and a standard mixture of the methyl esters of the isomers of linolenic acid were purchased from Sigma-Aldrich.

Several glass ampoules of length 4cm were made by cutting glass tubes with 5mm internal diameter and 1mm wall thickness. One of the ends of the glass tubes were sealed using propane-oxygen flame. Aliquots of pure methyl linolenate were introduced into the ready-to-seal glass ampoules. The other ends of the glass ampoules were melted and sealed by using propane-oxygen flame [16]. Three sets of glass ampoules each containing 15 tubes were grouped as A, B and C. The tubes in set A were placed in a chromatographic oven set at temperature 225˚C. Similarly, the other two sets B and C were placed at temperatures 250˚C and 275˚C respectively. The samples were removed at 1 hour (set A), 30minutes (set B) and 15 minutes (set C) time intervals. The samples removed were cooled and subjected to infrared and gas chromatographic analyses. Use of methyl linolenate will eliminate the need for derivatization after thermal induction. The methyl esters of the fatty acids in the mixture were analyzed by gas chromatography after proper dilution.

IR spectroscopic analysis:

A PerkinElmer Spectrum One FTIR spectrometer equipped with a Harrick single reflectance attenuated total internal reflectance (ATR) accessory and lead glycine sulphate detector was used in the IR spectroscopic analysis. A background spectrum was scanned in the range of 4000cm^{-1} to 600cm^{-1} prior to the scan of each sample. Each of the glass tubes was cut open at one of the ends and the sample was drawn with a capillary tube. The drawn sample was then spread over the ATR crystal. Then the infrared spectrum of the sample was measured and then converted to its second derivative profiles and saved for further analysis. The spectrum in the range of 1000 to 950 cm^{-1} was used for the identification of the *trans* isomer peak. Then the crystal was washed with acetone. The above procedure was repeated with all the samples.

Gas Chromatographic analysis:

GC analyses of Methyl linolenate isomers formed during the thermal induction were carried out by using Perkin Elmer, auto XL system gas chromatograph. A 120 m capillary column with 0.25 mm internal diameter coated with 0.25 µm thick, 70% cyanopropyl(equiv) polysilphenylene-siloxane stationary phase used. A

temperature program with initial temperature of 150˚C with 2 minutes equilibrium time, a temperature gradient of 0.5˚C/min up to 170˚C with 50 minutes holding time, then a temperature gradient of 1˚C/min up to 190˚C with 10 minutes holding time was used. The total running time was 122 minutes.

Isomerization kinetics:

Considering the isomerization as a single reaction of the type, **A** $\longrightarrow$**B+C+D** etc, where A is methyl linolenate and B, C, D etc. are isomers formed during the thermal reaction. The rate of the reaction for an n^{th} order chemical reaction can be determined as given below.

$$\frac{d[A]}{dt} = -k[A]^n \quad (1)$$

Where A is the concentration of initial compound, k is the rate constant for the reaction and n is the order of the reaction.

The equation (1) is rearranged as follows

$$\frac{d[A]}{[A]^n} = -kdt \quad (2)$$

The equation (2) is integrated for concentrations $[A]_t$ to $[A]_0$ and the results become

$$\frac{\{[A]^{(1-n)}\}_{A0}^{At}}{(1-n)} = -k[t]_0^t \quad (3)$$

Where $[A]_0$, $[A]_t$ are the concentrations of linolenic acid at time 0 and t respectively. The integral is valid for n≥2. When the order of the reaction is zero, then the equation (3) gives the relationship

$$[A]_0 - [A]_t = kt \quad (4)$$

For a reaction of first order the equation (3) gives the relationship

$$\ln\{\frac{[A]_t}{[A]_0}\} = -kt \quad (5)$$

It's clear from the equations that the determination of the kinetic parameters requires the precise determination of the concentrations of the reactants at time t. For a reaction of type first order, the rate of the reactions at temperature T_n can be calculated by graphics using equation (5).

Having calculated the rates of the reactions k_1, k_2 and k_3 for three different temperatures T_1 (225˚C), T_2 (250˚C) and T_3 (275˚C) the activation energy for the reaction can be determined by graphics using the Arrhenius equation (6).

$$k = Ae^{-\frac{Ea}{RT}} \quad (6)$$

Where A is the Arrhenius constant, E_a is the activation energy for the reaction, R is the universal gas constant and T is the temperature of the reaction.

Equation 6 can be re-written in the following form and a plot between lnk and 1/T would give the slope $-E_a/R$ and the activation energy E_a for the isomerization reaction can be calculated.

$$\ln k = \ln A - E_a/RT \quad (7)$$

RESULTS AND DISCUSSION

IR spectroscopy:

The infrared band assignments of edible oils are given in Table 2. An infrared spectra of methyl linolenate is shown in Fig. 1

Table 2: Some of the infrared band assignments of fats and oils.

Frequency (cm^{-1})	Functional group and mode of vibration
3025	=CH *trans* stretching
3006	=CH *cis* stretching
2953	CH (CH_3) asymmetric stretch
2924	CH (-CH_2-) asymmetric stretch
2854	CH (-CH_2-) symmetric stretch
1746	-C=O ester Fermi resonance
1653	-C=C- *cis* stretching
1465	-CH (-CH_2-, CH_3) bending
1377	-CH (CH_3) symmetric bending
1238	-C-O, -CH_2- stretching, bending
1161	-C-O, -CH_2- stretching, bending
1118, 1097	-C-O stretching
967	=CH *trans* stretching

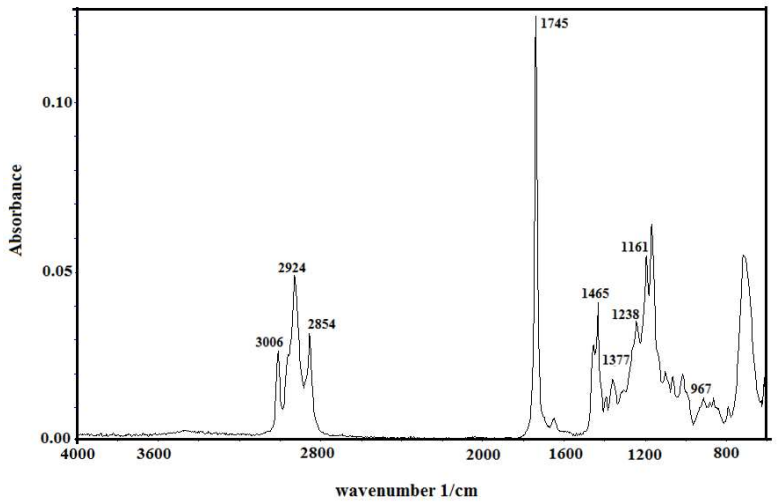

Fig. 1: Infrared spectrum of methyl linolenate

The second derivative spectral profiles of the IR spectra of thermally induced methyl-linolenate are shown in Fig. 2 a), b) and c) respectively.

The figures clearly show that the peak at 967cm^{-1} is increasing in intensity and indicating the formation of *trans* isomers of methyl-linolenate during the thermal induction. The *trans* CH bending of all the *mono, di* and *tri-trans* isomers of methyl linolenate absorb at 967cm^{-1}. The isomers with only *cis* configurations do not absorb in this region. The information obtained from the IR spectra is only qualitative and indicative of the formation of *trans* isomers. Determination of the concentrations of individual isomers using the absorption peak at 967 cm^{-1} is impossible. Furthermore, absorption arising from *cis*-CH stretching at 3006 cm^{-1} is a combination of cis stretching absorptions arising from the molecules ccc, cct, tcc, ctc, ctt, ttc and tct and quantitative measure of the remaining methyl linolenate in the mixture using this absotrption peak is also not possible. The determination of kinetic parameters for the isomerization reaction requires the precise determination of the concentrations of methyl linolenate in the mixtures. This can only be done by gas chromatography.

Gas chromatography:

The gas chromatographic analyses reveal the formation of all the isomers during the thermal induction. The major geometrical isomers formed in heat treatment of methyl-linolenate (9c12c15c) are identical to those identified by Wolff [17]. The identities of the isomers formed were confirmed by a gas chromatogram obtained with a standard mixture containing methyl derivatives of all the isomers of linolenic acid (Fig. 3).

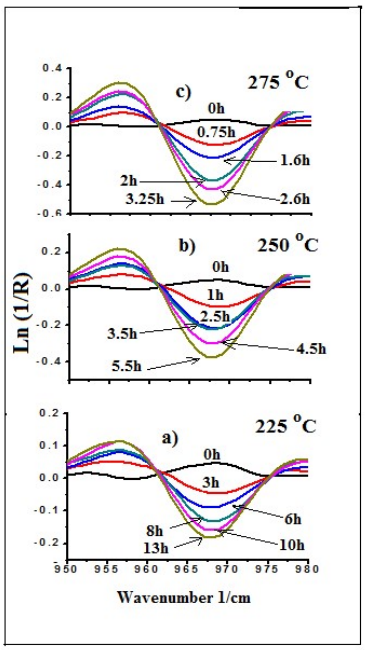

Fig. 2: Infrared spectra of pure and heat treated methyl linolenate samples

The formation and evolution of 18:3 geometrical isomers of methyl linolenate has been followed as a function of temperature and time of the reaction. The quantification of the concentrations of isomers in the heated mixtures was based on the relationship between the percentage calculated using peak height (sum of peak heights taken as 100 %) and concentrations (in %) in the standard mixture. Correction factors based on the real percentage composition versus percentage based on the peak heights for all the isomers were established and used in the quantification of the isomers in the mixture. The percentage composition by weight of the standard mixture as given by the vendor is shown in Table 3.

A collection of three chromatograms of the isomers formed during thermal treatment of methyl linolenate at 275 ºC is shown in Fig. 4. The results obtained (Fig: 4) seem to indicate that the *di-cis, mono-trans* trienes are present in small quantities in methyl-linolenate heat treated at 275˚C for 45 minutes. At this point only 6.2 % of methyl linolenate has transformed into its isomers. The *mono-cis, di-trans* trienes are in traceable quantities. After 3.75 hours of heat treatment at 275˚C, 63% of methyl-linolenate has transformed into its isomers. Most of the isomers of linolenic acid are present in the mixture. The chromatograms in Fig. 4 show clearly the formation of *mono-trans* isomers during the early stages of thermal induction. Then the *di-trans* isomers and finally the *tri-trans* isomer is formed.

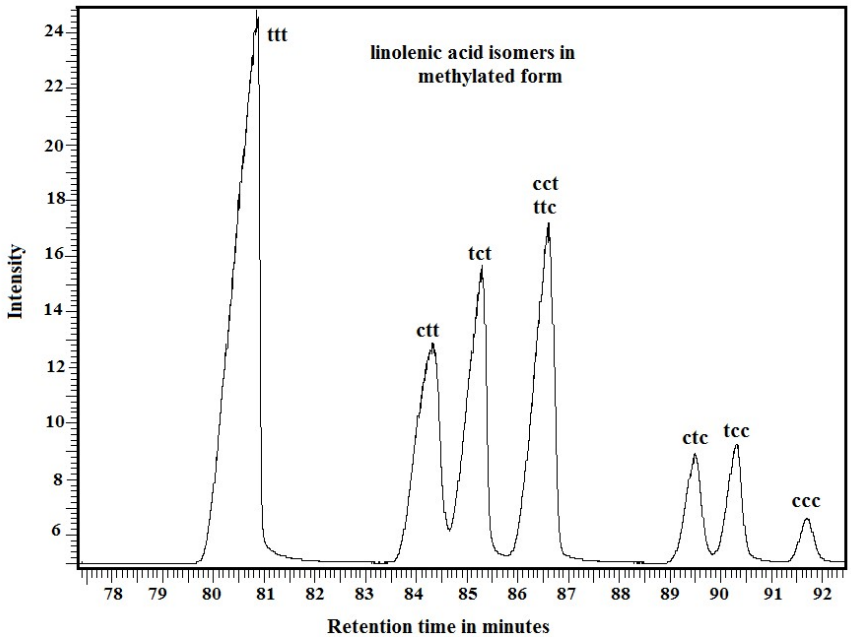

Fig. 3: Gas chromatogram of the reference standard of methyl linolenate isomers

Table 3: Composition of the methyl linolenate isomers in the standard mixture

Compound	Composition (%)
9c12c15c	3
9t12c15c	7
9c12t15c	7
9c12c15t	7
9t12t15c	15
9t12c15t	15
9c12t15t	15
9t12t15t	30

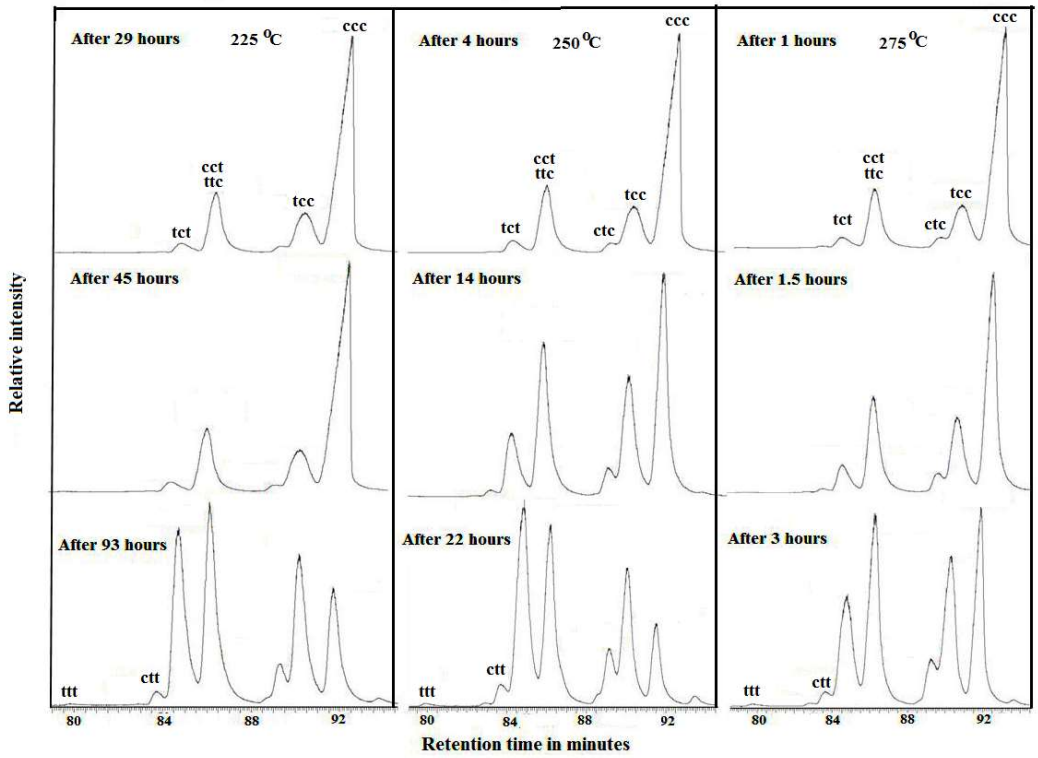

Fig. 4: A collection of chromatograms of the isomers formed during thermal treatment of methyl linolenate

Physical factors affect the ease of formation of certain *trans* isomers. Some *trans* isomers appear faster than the others for example *cct* and *tcc* isomers form faster than *ctc* isomer. Concentration differences of all the isomers in the heated mixtures can be justified by considering sterical factors in the methyl linolenate molecule.

The graphs obtained for the determination of rate constants k_1, k_2 and k_3 for the isomerization reactions at the three different temperatures (equation 3) are shown in Figure 5. Then the relationship between ln k and 1/T (equation 7) is shown in Figure 6. The results of the calculations are summarized in Table 3.

The activation energy determined for the isomerization reaction of methyl linolenate with respect to methyl linolenate is in the same range as the activation energies for the isomerization reactions in similar systems [10,11]. There are seven isomers formed during the isomerization of methyl linolenate. Two of them co-elute and determination of activation energies with respect to the individual isomers is rather difficult. Even after resolution of the co-eluting components, one needs to go through a series of complex mathematical analysis with the concentration profiles of the individual isomers before one can determine the activation energies.

The activation energy determined for the isomerization reaction of methyl linolenate is lower than the activation energy needed for the isomerization reaction of linoleic acid (39 k. cal/mol) and oleic acid (41 k. cal/mol) [9,11]. O'Keeffe et al. [18] determined activation energy (19.6±1.7 kcal/mol) for the isomerization reaction of linoleic acid in soybean oil with respect to linolenic acid. The results clearly illustrate the relative ease of the formation of *trans* isomers from linolenic acid. Fortunately, not many vegetable oils have high content of linolenic acid. It appears that most of the vegetable oils have relatively larger amounts of linoleic acid and therefore a large portion of *trans* fatty acids come from the contribution of isomerization reactions of linoleic acid in heated vegetable oils.

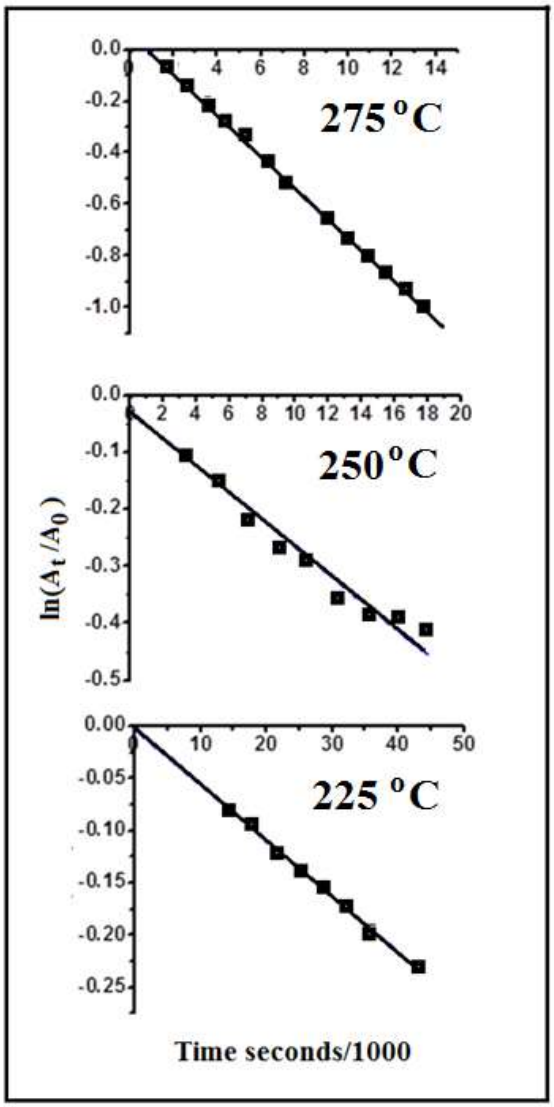

Fig. 5: Plots showing the correlations between ln[A_t/A_0] and time for the isomerization of methyl linolenate at temperatures 225,250 and 275˚C respectively

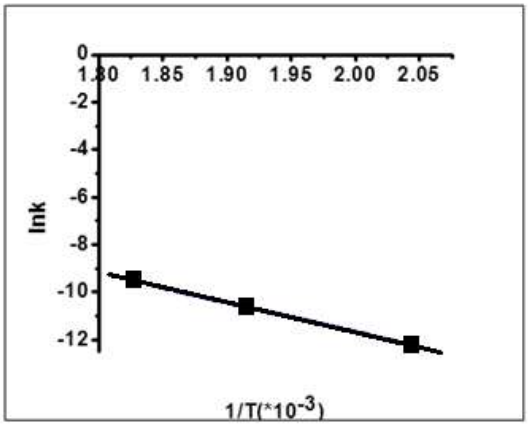

Fig. 6: A plot showing the correlation between ln k and 1/T

Table 3: Kinetic parameters for the isomerization reaction of methyl linolenate

Compound	Temperature (˚C)	Rate constant, k (s^{-1}) $K*10^{-3}$	Activation energy (kcal/mol)
	225˚C	0.0053±0.000	
Methyl-linolenate 9c12c15c	250˚C	0.0237±0.010	24.72±0.01
	275˚C	0.0790±0.010	

Conclusion:

In this study, it has been clearly demonstrated that Gas Chromatography can effectively be used in the determination of reaction rates and activation energy for the thermally induced isomerization of methyl-linolenate (9c12c15c). The isomerization reaction follows a first order kinetics with respect to the concentration of methyl-linolenate. Furthermore, the activation energy for the isomerization reaction is 24.72±0.01kcal/mol. We believe that the isomerization reaction of linolenic acid would have the same activation energy. The kinetic parameters determined in this work are in good agreement with values reported in the literature [18].

The results from the thermally induced isomerization experiments have revealed that the isomer 9c12c15c gradually decreases with time during the heat treatment and the *trans* isomers 9t12c15c, 9c12t15c, 9t12t15c, 9c12c15t, 9t12c15t, 9c12t15t and 9t12t15t gradually increase in the mixture of products. But, the rate of increase of individual isomer varies among them. The products are a mixture of almost all the isomers of 9c12c15c.

We have used a special experimental technique with glass ampoules for the study of isomerization kinetics. Only a few milliliter samples are required for the preparation of a series of samples for the analysis. Similar technique can effectively be applied to other compounds of same category.

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